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Review

Histone post-translational modifications and chromatin remodelers in colorectal cancer (CRC)

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List of abbreviations

cNUC Circulating nucleosome

CRC Colorectal Cancer

DNA Deoxyribonucleic acid

EZH2 Enhancer of zeste 2

HATi Histone acetyltransferases inhibitor

HATs Histone acetyltransferases

HDACi Histone deacetylases inhibitor

HDACs Histone deacetylases

HDMi Histone demethylases inhibitor

HDMs Histone demethylases

HMTi Histone methyltransferases inhibitor

HMTs Histone methyltransferases

JMJC Jumonji C

KMTs Lysine methyltransferases

LSD Lys-specific demethylase

MYC V-Myc Avian Myelocytomatosis Viral Oncogene Homolog

NuRD Nucleosome remodeling and histone deacetylase

PRMTs Protein arginine methyltransferases

PTMs Post-translational modifications

SMYD3 SET and MYND domain-containing protein 3

TSG Tumor Supressor Gene

Abstract

Colorectal cancer is the third most common type of cancer worldwide and the fourth most common cause of death. It is a disease that progresses through a series of steps, resulting from modifications in both genetic and epigenetic mechanisms. Despite significant advancements in treatment options, colorectal cancer patient survival is still poor owing to a lack of effective tools for early diagnosis and a limited capacity for optimal therapeutic decision making.

Growing evidence suggests an important role of epigenetic modifications in human cancer, including colorectal cancer, affecting every aspect of tumor progression, from initiation to metastasis. Epigenetic modifications related to DNA methylation have already been extensively studied in the context of cancer. However, the presence and clinical relevance of other modifications such as histone post-translational modifications and especially chromatin remodelers have only more recently started being explored and the results are still limited, but very promising.

The understanding of these mechanisms has contributed to the identification of new epigenetic biomarkers relevant to clinical practice, allowing for the optimization of diagnostic, prognostic and therapeutic response monitoring systems, as well as the generation of novel and targeted therapeutic approaches.

This review aims to present current understanding of the altered patterns of histone modifications and expression of chromatin remodelers in colorectal cancer, focusing on their potential clinical applications, especially the possible use of some of them as epigenetic biomarkers for early detection, management and risk estimation of colorectal cancer. The possibility of using epigenetic silencing as a therapeutic target in colorectal cancer is already being explored and will also be addressed here. Lastly, some challenges and future perspectives in this field of epigenetics will be presented, as a possible way to improve clinical outcome in patients with colorectal cancer.

Resumo

O cancro colo-retal é a terceira neoplasia maligna com maior incidência e a quarta causa mais comum de morte por cancro a nível mundial. É uma patologia que se desenvolve através de vários passos, resultando de alterações em mecanismos genéticos e epigenéticos. O prognóstico do cancro colo-retal é ainda bastante reservado, pela falta de ferramentas eficazes para diagnóstico precoce e pela capacidade limitada de terapêutica curativa.

Evidências crescentes apontam para um papel importante das alterações epigenéticas no cancro humano, incluindo o cancro colo-retal, afetando todos os aspetos do desenvolvimento tumoral, desde a iniciação, progressão e à metastização. Alterações epigenéticas associadas à metilação do DNA já foram extensamente estudadas no contexto do cancro. Contudo, a presença e relevância clínica de outras alterações como as modificações pós-traducionais de histonas e sobretudo a desregulação da expressão dos remodeladores da cromatina têm sido avaliadas apenas mais recentemente e os resultados são ainda limitados, mas promissores.

O aumento do conhecimento destes mecanismos tem contribuído para a identificação de novos biomarcadores epigenéticos com impacto na prática clínica, permitindo a otimização dos sistemas de diagnóstico precoce, prognóstico e monitorização da resposta terapêutica, bem como o desenvolvimento de novas abordagens terapêuticas mais dirigidas.

Neste artigo de revisão, será abordada a desregulação dos padrões de modificação de histonas e de expressão de remodeladores da cromatina no cancro colo-retal. Será dado especial ênfase ao potencial de aplicação dessas alterações na prática clínica, nomeadamente através da possibilidade de utilização de algumas delas como marcadores epigenéticos para deteção precoce do cancro colo-retal, avaliação da agressividade e do risco de metastização e estimação do prognóstico. Serão ainda avaliadas as potencialidades do recurso a silenciamento epigenético como alvo terapêutico no cancro colo-retal. Por último, será feita uma consideração sobre as perspetivas futuras neste campo da epigenética para a abordagem clínica do cancro colo-retal.

Keywords: colorectal cancer (CRC), epigenetics, histone post-translational modifications, chromatin remodelers, biomarkers, circulating nucleosomes

Methods

The writing of this review involved an extensive search of review and original articles published on the subject of epigenetics of colorectal cancer. This search was conducted online, using the free search engine PubMed. The following expressions were used to find relevant references: “histone modification in colorectal cancer”, “histone acetylation in colorectal cancer”, “histone methylation in colorectal cancer”, “chromatin remodelers in colorectal cancer”, “epigenetics in colorectal cancer”, “epigenetic biomarkers in colorectal cancer”. Some other expressions were used to conduct a more specific search to complement the findings in the articles retrieved with the more general search criteria. For example, the expressions “ARID1 colorectal cancer”, “EZH2 colorectal cancer” and “CHD5 colorectal cancer” were used to look for more recent works with these epigenetic markers. Additionally, some articles were also found through reading of previous reviews on the subject, including the ones by Huang et al. (2017), Gezer and Holdenrieder (2014), Jia and Guo (2013) and Bardhan and Liu (2013).

Articles that matched the search criteria and were clearly related to the theme of this review were selected according to their year of publication (only articles published after 2005 were included in this review, except in specific situations) and relevance to the aims of this review. Some importance was also given to the impact of these articles, measured by the number of citations. Articles were also preferentially selected according to the position in which they appeared in the search results in PubMed, when using the sorting option “Best Match”.

1. Introduction: CRC epidemiology and clinical management

Colorectal cancer (CRC) is the third most common type of cancer worldwide and the fourth most common cause of death (Haggar and Boushey, 2009). Globally, CRC is the third most commonly diagnosed cancer in males and the second in females, with 1,4 million new cases and almost 694 000 deaths estimated to have occurred in 2012 (Torre et al., 2012).The highest incidence rates are in Australia/New Zealand, Europe, and Northern America. Rates are low in Africa and South-Central Asia.

CRC clinical management can benefit from different strategies depending on its stage: health promotion through health education campaigns (when the disease is not yet present), the implementation of screening programs (for the detection of the disease in its early stages), and the development of nearly personalized treatments according to both patient characteristics (age, sex) and the cancer itself (gene expression) (Binefa et al., 2014).

Preventive measures for colorectal cancer include maintaining a healthy body weight, being physically active, minimizing consumption of red and processed meat and alcohol, and avoidance of smoking (Botteri et al., 2008; Ferrari et al., 2007; Giovannucci and Wu, 2006). Screening can detect colorectal polyps that can be removed before they become cancerous, as well as detect cancer at an early stage when treatment is usually less extensive and more successful. There are several accepted screening options (eg, the guaiac-based fecal occult blood test [FOBT], the immunochemical FOBT [or fecal immunochemical test], flexible sigmoidoscopy, stool DNA test, computed tomography [CT] colonography [“virtual colonoscopy”], double-contrast barium enema, and colonoscopy).

Despite advancements in treatment options, improvements in patient survival for CRC have been limited due to lack of early detection (Rawson and Bapat, 2012). Although some of the CRC screening tests available have proven to be effective in the reduction of incidence and mortality, a highly specific, noninvasive detection method has yet to be discovered. Molecular tests are expected to be more sensitive and specific than current methods. They will also provide genetic information about the malignancy in progression (Coppedè et al., 2014a).

The ideal molecular marker should have the following features: it should have high sensitivity and specificity; it should be safe and affordable so that it can be broadly accepted by patients; it should be easy to measure; and it should be consistently detected across genders and ethnic groups (Kumar, 2009; Huang et al., 2010; Parik and Vasan, 2007; Tanaka et. al, 2010). A robust biomarker should detect genomic/epigenomic alterations and/or variations in protein expression that specifically correlate to the disease helping clinicians make an accurate diagnosis.

Even if many potential biomarkers have been detected, the number of biomarkers that have been incorporated into clinical practice is surprisingly small (Choong and Tsafnat, 2012). Reliable biomarkers are needed to detect CRC in an early phase, to guide treatment, as well as for surveillance to detect recurrence and monitor therapeutic response.

2. Altered patterns of histone modifications and chromatin remodeling in CRC and their clinical relevance

In a eukaryotic cell, genes are coordinately activated or repressed to ensure cellular homeostasis (Nair and Kumar, 2012). In addition to a constant need to modulate the levels of expression of a large number of genes, mammalian cells are also faced with a topological challenge of packaging genetic information (about 2 metres of DNA) into the nucleus (Clapier and Cairns, 2009). An evolutionary solution to this problem is the unique ability of mammalian cells to package the DNA into higher order structures, commonly referred to as chromatin. The basic building blocks of chromatin are nucleosomes that are composed of 146 base pairs of DNA wrapped around an octamer containing two each of four core DNA packaging proteins - histones H2A, H2B, H3, and H4. The nucleosomes are further folded with the aid of linker histone H1 and non-histone proteins into an ordered, compact nucleoprotein complex (Saha et al., 2006;Thomas and Kornberg, 1975). Chromatin can be functionally divided into two subtypes, euchromatin and heterochromatin. Only about 1.5% of the human genome encodes for genes and very little of this coding sequence is present in heterochromatin (Babu and Verma, 1987).

2.1 Chromatin Remodelers

To fulfill cellular needs, genes that reside in euchromatin are continually undergoing regulated structural changes to allow for template-dependent processes like gene activation or repression. These functionally opposite events require dynamic packaging and unpacking of DNA elements, and the promoters and enhancers that control these events need to be exposed to provide access to regulatory factors and complexes. Hence, expression of genes must not only involve the general transcription machinery and specific transcription factors, but also largely depend on proteins capable of modifying the chromatin architecture. These unique proteins are called chromatin remodeling proteins or remodelers (Cairns, 2001, 2007).

By consuming ATP-derived energy, these complexes manipulate nucleosomal architecture through the dynamic mobilization (insertion/eviction) of nucleosomes as well as the configuration of nucleosomal DNA and histone octamers (Dawson and Kouzarides, 2012; You and Jones, 2012; Narlikar et al., 2013). Additionally, chromatin-remodelers recruit histone modifying proteins and numerous auxiliary proteins in order to construct large scaffolds, and participate in complex cross-talk networks with numerous transcription factors (Vaiopoulos et al., 2012).

To date, four remodeler families have been well characterized they include: SWI/SNF, ISWI, INO80 and NuRD/Mi-2. The four remodeler proteins share unifying properties and include (a) Ability to interact with nucleosome core, (b) Affinity for post-translationally modified nucleosomal histone-tail residues, (c) Core ATPase activity that utilizes energy from ATP hydrolysis to fuel remodeler functions, (d) Regulatory domains that are subject to various biochemical and epigenetic alterations, and (e) Specific protein domains and motifs that accommodate protein–protein interactions. Even though the remodeler proteins share these five basic properties they have evolved mechanistically to perform non-overlapping functions (Clapier and Cairns, 2009). Thus remodeler families are functionally specialized and have a unique role in regulating distinct transcriptional programs in response to cellular cues.

Chromatin-remodeling complexes orchestrate a wide range of physiological processes including, proliferation, self-renewal and differentiation (Vaiopoulos et al., 2012). Since transcriptional control is essential for living processes, it is not surprising that genetic alterations in chromatin remodeling components are intimately linked with cancer and chromatin remodelers can function as tumor suppressors or promoters depending on contextual signaling (Jones and Baylin, 2007; Shain et al., 2012).

A large number of genomic loci that are repressed in the physiologic state are activated by an aberrant expression and/or activity of remodelers in cancerous cells in response to deregulated oncogenic signals (Ellis et al., 2009). Therefore, deregulation of chromatin leads to altered gene activation and/or inappropriate gene silencing. Several tumor suppressors such as retinoblastoma (pRb), p53 and Ini1/hSNF5 which utilize chromatin remodeling as part of its normal functions are misregulated in certain cancers (Gregory and Shiekhattar, 2004; Hickman et al., 2002).

In colorectal cancer, nucleosomal insertion in the promoter CpG island region may facilitate epigenetic gene silencing through heritable modifications in chromatin conformation, as in the case of MLH1 (Lin et al., 2007). Similarly, distant Wnt-related responsive enhancers may reach and up-regulate c-Myc, a pivotal coordinator of proliferation and apoptosis in CRC, through the generation of large chromosomal loops (Yochum, 2011). More recent experimental data link the upregulation of vascular endothelial growth factor (VEGF) by peroxisome proliferator

activated receptor δ (PPAR-δ) through a mechanism that involves β-catenin-mediated chromatin looping (Hwang et al., 2012).

Overexpression or mutated forms of several members of the remodeling machinery have been identified in a broad range of hematological and solid malignancies, including CRC.

SWI/SNF chromatin remodeling complex regulates critical cellular processes including cell cycle progression, programmed cell death, differentiation, genomic instability and DNA repair (Narlikar et al., 2013). The SWI/SNF complex, containing one of 2 mutually exclusive ATPase subunits, BRG1/SMARCA4 or BRM/SMARCA2, physically alters nucleosome positioning using energy generated by ATP hydrolysis (Jaclyn et al., 2014). The complexes can be subdivided into two broad categories, BAF or PBAF, based upon the presence of the ARID1A/B subunits, or ARID2 and PBRM1 subunits, respectively (Kadoch et al., 2013; Wei and Weissman, 2014).

In CRC, SWI/SNF complex seems to be involved in multiple aspects of development and progression. For instance, BRG1 is implicated in the promotion of metastasis through a mechanism involving the down-regulation of E-cadherin (Sanchez-Tillo et al., 2010). Expression of BRG1, but not BRM, was found to be frequently elevated in CRC specimens by Watanabe et al. (2011). They also found that PTEN expression was negatively regulated by BRG1, which subsequently influenced the cyclin D1 levels via the PI3K–Akt signalling pathway. Shengtao et al. (2016) showed increased expression of BRG1 was associated with AJCC stage, depth of tumor invasion, distant metastasis, and histologic differentiation in colon cancer. These results indicated that BRG1 is closely correlated with aggressive malignant behavior and its presence predicts poor survival for colon cancer patients. BRG1 failed to act as an independent prognosis predictor on multivariate analysis but, in collaboration with other biomarkers, BRG1 expression levels could be taken into consideration when making treatment decisions. Patients with elevated BRG1 expression require more powerful adjunctive treatments and frequent postoperative follow-up, which serves as a profound guide in clinical treatment. Additionally, Zhu et al. (2016) demonstrated the important roles of the BRG1/STAT3/VEGFC in tumor-associated lymphangiogenesis, which might lead to the discovery of novel therapeutic targets in the treatment of CRC.

Inactivating mutation of several other SWI/SNF members is also a frequent event in CRC patients (especially with MSI), including ARID1A (Network, 2012) and SMARCC2 (Kim et al., 2013). All the 15 genes of the ARID gene family contain the ARID domain, which is known to be centrally involved in chromatin regulation (Patsialou et al., 2005; Wang et al., 2004). Frequent inactivating mutations in ARID1A have been identified in a wide variety of cancers, including CRC, and it has been recognized as a novel tumor suppressor gene (Wu and Roberts, 2013). As already shown by Jones et al. (2012), Cajuso et al. (2014) confirmed that ARID1A is frequently mutated also in MSI CRC. Their results further suggested that, based on their mutation frequency, ARID1B, ARID2 and ARID4A should be considered new candidate genes for MSI CRC. Lee et al. (2016a) showed that cases with ARID1A loss were associated with poor differentiation, lymphovascular invasion and higher pT stage. However, at a median follow-up of 49 months, ARID1A loss did not correlate with overall, disease-specific, or recurrence-free survival. These data indicated that ARID1A loss lacks prognostic significance in the studied population, despite its association with other adverse features. Another study, however, concluded that, specifically among the mismatch repair(MMR)-deficient cases, ARID1A loss correlated with medullary histology and an increased rate of nodal and distant metastasis and these patients also tended toward a worse 5-year overall survival (Ye et al., 2014).

SMARCB1 is also a core component of the BAF (hSWI/SNF) complex and its negative expression (found in 11% of CRCs) was associated with poor differentiation, liver metastasis and shorter patients' survival regardless of the MMR status or tumor stage (Pancione et al., 2013).

CHD5 belongs to a group of SWI/SNF proteins called chromodomain helicase DNA binding (CHD) proteins, which contain a SWI/SNF-like/ATPase domain, and some members form a nucleosome remodeling and deacetylation complex that directly modifies chromatin structure and enhances the binding of transcription factors to their binding sites (Thompson et al., 2003). As such, CHD5 has an impact on gene regulation, and it was shown to be highly methylated in colon cancer tumors of African American patients (Mokarram et al., 2009). Fatemi et al. (2015) showed that the CHD5 gene is repressed in all types of adenomas, either epigenetically or by chromosomal deletion. As such, CHD5 likely acts as a tumor-suppressor gene in early colorectal carcinogenesis.

Moreover, other studies suggest that the expression of other members of the chromodomain helicase/adenosine triphosphate-dependent chromatin remodeling family was also altered in CRC. Tahara et al. (2014) showed that genes that regulate chromatin were mutated in CIMP1 CRCs and the highest rates of mutation were observed in CHD7 and CHD8. Kim et al. (2011) detected mutations in the CHD8 gene in 28.6% of the MSI-H CRCs, with loss of expression.

Another chromatin-remodeler, nucleosome remodeling and histone deacetylase (NuRD), determines the fate of various signaling pathways (Vaiopoulos et al., 2012). NuRD is responsible, at least in part, for the repression of the transcriptional activity of AP-1 transcription factor. AP-1 possesses a pivotal role in the orchestration of intestinal proliferation and the promotion of tumorigenesis (Aguilera et al., 2011). NuRD however is also involved in the silencing of tumor suppressor genes, such as negative regulators of Wnt, often in synergy with DNMTs (Cai et al., 2013). In accordance to this data, immunohistochemical analysis of surgically resected colorectal tumors correlated the positive expression of HDAC1 and MTA1, two NuRD members, with decreased overall survival and poor clinical outcome (Higashijma et al., 2011). Cai et al. (2014) established an oncogenic role for CHD4, another NuRD component, for initiating and supporting tumor suppressor gene (TSG) silencing in human colorectal cancer. In their study, CHD4 knockdown activated silenced TSGs, revealing their role for blunting colorectal cancer cell proliferation, invasion, and metastases. In another study, high CHD4 levels plus low expression of TSGs strongly correlated with early disease recurrence and decreased overall survival. Since CHD4 has ATPase activity, this data identified CHD4 as a potentially novel drug target in cancer (Xia et al., 2017).

Special AT-rich sequence-binding protein 1 and 2 (SATB1/2) are nuclear matrix-associated proteins also involved in chromatin remodeling and regulation of gene expression. Mansour et al. (2016) concluded that SATB1 expression was increased, whereas SATB2 expression was reduced, in colorectal cancer tissues compared to control tissues. Brocato et al. (2015) presented four major conclusions regarding the role of these proteins in CRC and their potential clinical value as biomarkers in CRC: (i) SATB2 is a sensitive marker to distinguish CRC from other cancer types, (ii) Reduced expression of SATB2 in CRC is associated with poor prognosis, (iii) High levels of SATB1 expression facilitate CRC and are associated with poor prognosis.

The Tip60 histone acetyltransferase is implicated in DNA-damage response, exerts anti-proliferative effects and represses the Wnt-pathway. It belongs to a multi-molecular complex that contains many chromatin remodeling enzymes including the ATPase p400, a protein involved in nucleosome incorporation of specific histone variants and that can directly or indirectly repress some Tip60-dependent pathways, up-regulating Wnt. Tip60 activity is critical for the cellular response to DNA damage and is affected during cancer progression. Mattera et al. (2009) found that the ratio between Tip60 and p400 mRNAs is affected in most colorectal carcinoma and is critical for the response to 5-fluorouracil, a first-line treatment against colon cancer. These results were supported by Chevillard-Briet et al. (2013), which confirmed that, In CRC, the ratio of Tip60/p400 is unbalanced in favor of p400 and promotes Wnt activity and carcinogenesis. The results of another study suggested that Tip60 was more frequently down-regulated in advanced colorectal carcinoma and could serve as a potential marker for the malignancy of CRC (Sakuraba et al., 2009)

HELLS (also known as SMARC6 and LSH) gene encodes a protein related to the SNF2 family of chromatin-remodeling ATPase. This protein is essential for correct establishment of DNA methylation level and for efficient repair of DNA double-strand breaks. Choi et al. (2015) observed aberrant bands of the HELLS gene in eight cancers (seven CRC and one GC). DNA from normal tissue showed no shifts in SSCP (single-strand conformation polymorphism), indicating the aberrant bands had risen somatically, indicating a possible role of HELLS as a TSG in CRC.

One study also revealed that BPTF, a chromatin remodeling-related gene, exhibits frameshift mutations in gastric and colorectal cancers (Lee et al., 2016b).

Table 1 (see Annex I) presents a summary of the chromatin remodelers found to have an altered expression in CRC and possibly associated with tumorigenesis and, in some cases, also associated with clinical outcomes.

2.2 Histone post-translational modifications

In addition to ATP-dependent chromatin remodelers, the core nucleosome histone structures have a unique role in determining chromatin architecture. Interaction of histone proteins H2A, H2B, H3 and H4 with nucleosome DNA has a special relevance in chromatin biology as these interactions regulate an overall affinity of nucleosome histones with DNA, leading to a relaxed or condensed chromatin.

The four core histones are subject to a plethora of post-translational modifications that are inscribed by histone-modifying enzymes and multiple modifications, such as phosphorylation, acetylation, methylation, ubiquitination and citrulation (Peterson and Laniel, 2004). While it is believed that post-translational modifications of histone-tails and residues within globular histone can alter nucleosome mobility, some studies have demonstrated that a combinatorial pattern of histone modification may constitute an epigenetic code to be recognized, interpreted or modified by other chromatin binding proteins with specialized histone binding domains (Berger, 2002). Binding of effector proteins to modified histone modules are important for full transcriptional outcomes. It has been demonstrated that the

epigenetic language, or “histone code”, acts as a molecular beacon and might offer a bird's eye view of the chromatin landscape for transcription factors and coregulatory proteins with roles in gene expression or repression (Strahl and Allis, 2000). Therefore, histone's post-translational modifications increase capacity of genome to store and/or transmit biologic information.

Modifications of histone tail in the steady or dynamic-state of chromatin are tightly controlled by histone modifying enzymes and perturbations in such components are often associated with cancer. In contrast to methylation-associated alterations, the understanding of histone modification patterns in human cancers and their correlation with the CIMP status and the different CRC pathways is limited (Goel and Boland, 2012) and the best studied histone modifications in CRC are acetylation and methylation of lysine and arginine residues within histone tails.

2.2.1- Histone acetylation: players and patterns

Histone acetylation is reversible modifications of lysine residues on histone “tails” and is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) that typically act as transcriptional co-activators or co-repressors, respectively. Of all the known modifications, acetylation has the most potential to unfold chromatin since it neutralizes the basic charge of the lysine (Coppedè, 2014b). HATs are divided into three main families: GNAT, MYST and CBP/p300. Most of the acetylation sites characterized to date fall within the N-terminal tail of histones, which are more accessible for modification. The reversal of acetylation correlates with transcriptional repression. There are three distinct families of HDACs: the class I and class II HDACs and the class III NAD-dependent enzymes of the Sirt family (Weichert et al., 2008). They are involved in multiple signaling pathways and they are present in numerous repressive chromatin complexes. In general, these enzymes do not appear to show much specificity for a particular acetyl group. In cancer cells, disruption of the balance between HATs and HDACs contributes to transcriptional inactivation of tumor suppressor genes (TSGs) (Fraga et al., 2005; Seligson et al., 2005).

Several investigators observed global H3 and H4 lysine hypoacetylation in CRC cell lines and primary tumors, including CRC, often associated with downregulation of tumor suppressor genes and metastasis suppressor genes (Gargalionis et al., 2012). Fraga et al. (2005) found global loss of H4K16ac in cancer cells and primary tumors, including colonic tumors. Subsequent studies investigated the global pattern of individual histone marks, mainly by immunohistochemistry. Two different studies reported that global levels of H4K12ac and H3K18ac increased in adenocarcinomas in respect to normal tissue or adenoma (Ashktorab et al., 2009; Nakazawa et al., 2012). Acetylation of H3K9 (H3K9ac) correlated with the tumor histological type (Tamagawa et al., 2012).

Alterations in HDACs are found in many human cancers including CRC (Marks et al., 2001). Multiple class I HDACs are up-regulated in a subset of CRCs, including HDAC1 in 36.4%, HDAC2 in 57.9%, and HDAC3 in 72.9% of CRC specimens, and high HDAC2 expression has been shown to be associated with reduced patient survival in CRC (Ashktorab et al., 2009). In particular, HDAC2 upregulation was found early in colon field carcinogenesis (Stypula-Cyrus et al., 2013). Nuclear expression of HDAC2 was observed in 81.9% of colorectal carcinoma, 62.1% colorectal adenoma and 53.1% of normal tissues, respectively (Ishihama et al., 2007). The overexpression of HDAC2 was found to be accompanied by the hypoacetylation at H4K12 and H3K18 histones during adenoma to carcinoma progression, contrarily to what was observed in other studies. Upregulation of several class II (HDAC5 and HDAC7) and class III (sirtuins) HDACs has also been observed in CRC, and linked to downregulation of genes involved in the Wnt signaling pathway (Rönsch et al., 2011; Stypula-Cyrus et al., 2013). HDAC4 missense mutations have been observed and HDAC4 has been shown to be downregulated in colon cancers (Bassett and Barnett, 2014). In CRC, Bassett and Barnett (2014) also reported an HDAC5 downregulation but an HDAC7 overexpression. The class III HDAC, SIRT1, is shown to be overexpressed in 40% of CRCs associated with CIMP-high and MSI-H tumors in a study of 485 CRC cases (Nosho et al., 2009). Inhibition of SIRT1 can reactivate the transcriptionally silenced genes, suggesting a possible therapeutic approach to reverse epigenetic alteration-induced silencing process of many genes (Strahl and Allis, 2000).

CREB-binding protein (CBP)/p300 and their associated factor (PCAF) facilitate the effects of β-catenin through acetylation (Chevillard-Briet et al., 2013). Strikingly, the role of p300 in CRC has not yet been elucidated as its overexpression has been associated with advanced tumor stages, metastasis and poor clinical outcome (Ishihama et al., 2007), while other research groups have linked p300 expression with increased survival (Huh et al., 2013). p300 binds to and acetylates MTA2, a member of metastasis associated family proteins, and this is seems to be important for CRC cells growth and migration (Zhou et al., 2014).

Human MOF (males absent on the first) is a member of the MYST (Moz-Ybf2/Sas3-Sas2-Tip60) family of histone acetyltransferases (HATs). As a catalytic subunit, MOF can form at least two distinct multiprotein complexes (MSL and

NSL) in human cells. Both complexes can acetylate histone H4 at lysine 16 (H4K16); however, the NSL complex possesses broader substrate specificity and can also acetylate histone H4 at lysines 5 and 8 (H4K5 and H4K8). Silencing of MOF in cells leads to genomic instability, inactivation of gene transcription, defective DNA damage repair and early embryonic lethality. Unbalanced MOF expression and its corresponding acetylation of H4K16 have been found in certain primary cancer tissues, including colorectal carcinoma (Su et al., 2016).

2.2.2- Histone methylation, players and patterns

Histone methylation is other most frequent post-translational modification that occurs both on lysine and arginine residues of the N-terminal tail of histones. Like histone acetylation, histone methylation is now appreciated as a reversible process (Bannister et al., 2011). Its homeostasis is mediated by two antagonizing groups of enzymes, histone methyltransferases (HMTases) and histone demethylases (HDMs), which install and remove histone methylation marks, respectively, in a site-specific manner (Klose and Zhang, 2007). To date, more than twenty lysine and arginine HMTases have been identified. Each of the HMTases, either acting alone or in complex with other HMTases, catalyzes site-specific histone methylation. For histone H3, methylation has been observed at multiple lysine sites, including H3K4, K9, K27, K36 and K79, and addition of up to three methyl groups at each lysine produces a total of four methyl states: unmethylated, monomethylated, dimethylated and trimethylated. H3K4 trimethylation (H3K4me3) is strongly associated with transcriptional competence and activation, with the highest levels observed near transcriptional start sites of highly expressed genes. Two other methylation sites on histones, H3K36 and H3K79, are implicated in activation of transcription (Vakoc et al., 2006). On the contrary, H3K27 trimethylation (H3K27me3) is frequently associated with gene silencing (Barski et al., 2007). Two other lysine methylation sites that are connected to transcriptional repression are H3K9 and H4K20 (Kouzarides, 2007). The establishment of an appropriate pattern of histone methylation is not only crucial for normal development and differentiation, but is also intimately associated with tumor initiation and development. Histone methylation at key lysine residues has been shown to work in concert with acetylation and other modifications to provide a histone code that may determine heritable transcriptional states (Jenuwein and Allis, 2001).

Changes in histone lysine methylation status have been observed during CRC formation, which is thought to be a consequence of dysregulation of histone lysine methyltransferases or the opposing demethylases (Berry and Janknecht, 2013). It has been shown that several of these changes in methylation status correlate with pathological features of CRC, such as tumor size, histological type and tendency to lymph node invasion and distant metastasis. Methylation of histone tails has been largely documented in CRC primary tumors and cell lines, including loss of tri-methylation of H4K20, and di- and tri-methylation of H3K9 (H3K9me2/me3), H3K4 (H3K4me2/me3) and H3K27 (H3K27me2/me3)(Gargalionis et al., 2012; Fraga et al. 2005) found global loss of H4K20me3 in cancer cells and primary tumors, including colonic tumors. Later studies revealed that H3K9me2 expression was associated with the progression of adenoma to adenocarcinoma. The global level of H3K9me2 was distinctly higher in neoplastic cells (adenoma and adenocarcinoma) than in normal glandular cells; in addition, it was significantly higher in adenocarcinoma than in adenoma and was specifically increased in invasive regions of CRC tissues (Yokoyama et al., 2013). Moreover, the presence of H3K9me3 positively correlated with lymph node metastasis in patients with CRC (Nakazawa et al., 2012). However, H3K9me3 and H4K20me3 have been found to be almost absent at the pericentromeric satellite II repeat in patients with CRC when compared with healthy controls or patients with multiple myeloma (Gezer et al., 2011; Leszinski et al., 2012a). More recently, through next-generation sequencing of immunoprecipitated plasma DNA, (Gezer et al., 2013) confirmed reduced levels of H3K9me3 and H4K20me3-related repetitive sequences in circulation of patients with CRC. Consistently, high expression of H4K20me3 and H3K9me3, and low nuclear expression of H3K4me3, were associated with good prognosis in early-stage CRC patients (Benard et al., 2014).

As a well-known gene activation marker, H3K4me3, was found to be elevated in tumor tissue of CRC patients and several cell lines, resulting in activated expression of WNT-signaling target genes through interaction between SET Domain containing 1A (SETD1A) and of β-catenin (Salz et al., 2014). Interestingly, H3K4me1/2/3 were all decreased at the MutL Homolog 1 (MLH1) promoter in SW480 cells under hypoxia, leading to silence of MLH1 and DNA mismatch repair defects, a key process in the development of sporadic CRC (Lu et al., 2014). Additionally, dimethylation of H3K4 (H3K4me2) correlated with the tumor histological type and lower levels of H3K4me2 correlated with a poor survival rate. The multivariable survival analysis showed that H3K4me2 status is an independent prognostic factor for patients with CRC (Tamagawa et al., 2012). The transcription-repression marker H3K27me3 was found to be increased in tumor tissue of CRC patients with poor prognosis (Benard et al., 2013, 2014). In addition, it has been found that the methylation level of H3K27me2 detected with immunohistochemistry is an independent prognostic factor for metachronous liver metastasis of colorectal carcinomas (Tamagawa et al., 2013).

Lastly, dimethylation of histone 3 lysine 79 (H3K79me2) was elevated in CRC patients with poor prognosis, enhancing IL-22-induced cancer stemness (Kryczek et al., 2014).

Among the writers and erasers of these methylation marks, the polycomb repressive complexes PRC1 and PRC2 mediate epigenetic silencing of several tumor suppressor genes in cancers via histone methylation. PRC1 binds at H3K27me3 and possesses histone-ubiquitylation abilities. The histone methyltransferase enhancer of Zeste 2 (EZH2) is the central unit of PRC2, responsible for H3K27me3 (Simon and Kingston, 2009). PRC1 and PRC2 may cooperate for gene repression or act in an independent manner (Varier and Timmers, 2011; Richly et al., 2011). Polycomb proteins coordinate crucial developmental steps and their malfunction is often observed in various malignancies. Both PRC1 and PRC2 interact with DNMTs and are believed to play a fundamental role in transcriptional gene silencing in CRC (Ohm et al., 2007). In vitro evidence from CRC cells indicate the involvement of EZH2 in tumor proliferation and growth (Fussbroich et al., 2011). The PRC protein EZH2 is frequently found to be overexpressed in various solid tumors, including colon cancer (Simon and Lange, 2008; Bracken and Helin, 2009; Liu et al., 2015). The oncogenic function of EZH2 has been mechanistically attributed to the silencing of tumor suppressor genes, including INK4B-ARF-INK4A, p57 ^{KIP2}, p27 (Gonzalez et al., 2009), BRCA1 (Yu et al., 2007) and adrenergic receptor β2 (Natarajan et al., 2010). PRC1 and PRC2 complexes are implicated in metastasis through their ability to repress E-cadherin via B lymphoma Mo-MLV insertion region 1 homolog (Bmi1) and EZH2 respectively (Lee et al., 2012). Bmi1 and other members of the polycomb group family have been found elevated in CRC patients and have been correlated with advanced stages and aggressive types of carcinomas (Du et al., 2010).

Recent experimental data reveal other links between HMTs and key regulatory signaling pathways. KMT2B/ MLL4, KMT2D/MLL2, and SETD1A are all H3K4 methyltransferases that promote the development of CRC (Denissov et al., 2014; Nguyen et al., 2008; Nightingale et al., 2007). MLL2, an epigenetic regulator in mammalian cells, mediates histone 3 lysine 4 tri-methylation (H3K4me3) through the formation of a multiprotein complex. Elevated MLL2 expression level has been observed in poorly differentiated and more invasive colon carcinoma cell lines, and in colon carcinoma tissues as compared to the corresponding adjacent normal colonic epithelium (Kang et al, 2007). MLL2 is primarily regarded as a nuclear protein with nuclear localization signal. Colonic cell lines derived from highly invasive tumors, exhibited altered sub-cellular distribution and proteolytic processing of MLL2 compared to the non-tumor/less invasive tumor cell lines. Mixed lineage leukemia-4 (MLL4) regulates the expression of various cyclins, p-proteins and HOX genes that are critical players in cell-cycle progression, via histone H3K4 trimethylation in the target gene promoters (Ansari et al., 2012). Natarajan et al. (2010) showed that knockdown of MLL4 resulted in cell-cycle defect and induced apoptosis in colon cancer cells. Further, MLL4/KMT2D was found to be significantly elevated in tumor tissue compared with adjacent benign mucosa in CRC patients' samples and cell lines. Levels of SETD1A were elevated in human CRC cells and patient samples; while depletion of SETD1A inhibited CRC cell growth and affected about 50% WNT target genes (Salz et al., 2014).

Interestingly, H3K4 demethylases, KDM1A/LSD1 and KDM5B/JARID1B (Secombe and Eisenman, 2007), like H3K4 HMTs, also promote the development of CRC, suggesting that the local H3K4 methylation profile might be better associated with CRC. JARID1B is involved in CRC maintenance, and depletion of JARID1B led to loss of epithelial differentiation and suppression of CRC cell growth (Ohta et al., 2013). Lysine specific demethylase 1 (LSD1) seems to potentiate proliferation, invasiveness and metastatic potential of CRC cells (Ding et al., 2013). LSD1 has been positively correlated with TNM stage, lymph node infiltration and metastatic disease in CRC patients (Jie et al., 2013).

SUV39H1 is an HMTase, which specifically methylates H3K9 at the pericentric heterochromatin. Tiwari et al. (2008) found elevated SUV39H1 expression level in 54 of 219 CRC patients specimens examined. EHMT2/G9a is also responsible for dimethylation of H3K9 (H3K9me2). Recently, EHMT2 was found to be much higher expressed in CRC tumor tissue than peritumoral counterparts, with a possible role in CRC tumorigenesis (Zhang et al., 2015b).

Takada et al. (2007) noticed that NLK, known to antagonize WNT signaling, phosphorylates SETDB1, a histone methyltransferase, leading to the formation of a corepressor complex that inactivates the activity of the transcriptional factor PPARγ through histone 3-K9 methylation in CRC. This suggests that NLK and SETDB1 affect Notch-mediated inhibition of WNT target gene expression by modifying histone status in CRC in this tumor type (Kim et al., 2012).

KDM4B and KDM4C are both demethylases of H3K9. High expression of KDM4B was correlated with lymph node status, Duke's classification and tumor invasion of CRC patients (Liu et al., 2013). Consistent with this finding, KDM4B was upregulated in colon and rectal adenocarcinomas (Berry et al., 2014). KDM4C overexpression was found in colon cancer cell lines, whereas KDM4C downregulation associated with reduced growth and clonogenic capacity of colon cancer cells (Kim et al., 2014). These results support both KDM4B and KDM4C as oncoproteins in CRC.

DOT1L is an activator of the Wnt-dependent transcription in CRC cells (Simon and Lange, 2008). DOT1L is the only non-SET-domain-containing PKMT that methylates H3K79 (Steger et al., 2008). High expression of DOT1L in CRC tissue is a predictor for poor prognosis (Kryczek et al., 2014).

Other HMTs overexpressed in CRC include: SMYD3, the methyltransferase of H4K5 (Van Aller et al., 2012); WHSC1/MMSET/NSD2, responsible for the methylation of H4K20, and whose expression level was correlated with tumor aggressiveness (Hudlebusch et al., 2011); CARM1/PRMT4, which methylates H3R17 and H3R26 (Ou et al., 2011); PRMT5, which catalyzes symmetric dimethylation on histone 3 arginine 8 (H3R8me2s) and histone 4 arginine 3 (H4R3me2s) and induces transcriptional repression and has been associated with poor patient survival (Zhang et al., 2015a).

Recently, direct mutations in histone-methylation sites have also been found to contribute to abnormal histone-methylation profile, then cancer development. Histone 3 lysine 36-to-methionine (H3K36M) mutation was identified in a CRC sample (Shah et al., 2014). This mutation has been proved to impair mesenchymal progenitor cell differentiation and promote undifferentiated sarcoma in vivo (Lu et al., 2016), suggesting that H3K36 methylation is an important epigenetic marker for tumor suppression.

Apart from the widely studied histone modifications, ubiquitination may serve as an alternative mechanism of gene regulation in CRC. Histone ubiquitination can be associated with both gene silencing and activation. It has been shown that an H2B ubiquitin ligase is mutated in various solid tumors including CRC, whereas an H2B deubiquitinase has been reported to be overexpressed in colorectal and breast cancer (Johnsen, 2012). Recently, loss of H2B monoubiquitination has been linked with the abnormal cancer cells' glucose metabolism, which permits survival in adverse conditions (Urasaki et al., 2012). In vitro and in vivo data point out that histone 2A ubiquitination, mediated by the PRC1 complex member Bmi1, is actively involved in the proliferation of CRC cells (Yu et al., 2012).

In colorectal cancer a high phosphorylated histone H2AX (γH2AX) expression in CRC tissues was associated with tumor stage and perineural invasion. Furthermore, a high γH2AX expression was associated with poor distant metastasis-free survival (DMFS) and OS. A high γH2AX expression in CRC tissues is associated with a more malignant cancer behavior, as well as poor patient survival (Lee et al., 2015).

Table 2 (see Annex I) presents a summary of the histone post-translational modifications and respective regulatory enzymes found to be altered in CRC and possibly associated with tumorigenesis and, in some cases, also associated with clinical outcome.

3. Epigenetic-based markers for CRC detection, management and risk estimation: some considerations

A sensitive and specific diagnostic marker is not only useful in early diagnosis, but also helps in assessing the risk of developing the disease. Advances in the technology have enabled investigators to isolate metabolites, proteins and DNA from body fluids and fecal material and correlate them with pathophysiological symptoms of diseases including cancer, uncovering a battery of markers for cancer diagnosis; however, only few could reach to clinics because of issues of sensitivity and specificity. Therefore, at one side there is a need to improve techniques and on the other hand discovery of new markers is of immense importance. Presence of histone proteins is not known in fecal and urine samples; therefore, histone posttranslational modifications have been utilized as cancer diagnostic markers using circulating nucleosomes (cNUCs) in serum samples (Matei and Nephew, 2010). A blood-based, minimally invasive test is seen as a highly attractive approach to increase screening compliance and CRC detection.

Nucleosomes are released by apoptotic and necrotic cells into the blood circulation. Although macrophages efficiently clear dead cells by phagocytosis (Jiang et al., 2003), nucleosomes might enter the circulation reflecting either increased production or impaired clearance. In addition to apoptotic and necrotic processes, the active release of DNA from all living normal and diseased cells into the bloodstream has also been described (Stroun et al., 2000). In patients with cancer, the release of nucleosomes and DNA is elevated due to increased cell turnover (Schwarzenbach et al., 2011). Sera of patients with malignant tumors including colorectal cancer showed high levels of nucleosome compared with those of healthy persons and patients with benign diseases (Holdenrieder et al., 2001).

As nucleosomes are stable structures in the circulation (Holdenrieder et al., 2010), they might be a valuable source of novel biomarkers (Rahier et al., 2017). However, the diagnostic potential of histone tail modifications in CRC is limited if compared with DNA methylation and noncoding RNA biomarkers that can be much more easily

detected in circulating blood DNA. Even so, data on the utility of detecting of methylation marks on circulating nucleosomes as a novel cancer biomarkers is accumulating.

Through chromatin immunoprecipitation-associated quantitative PCR, in 2008, the detection of histone methylation in blood circulation was carried out (Deligezer et al., 2008). Two histone methylation marks, H3K9me3 and H4K20me3, the hallmarks of pericentric heterochromatin (Barski et al., 2007), were investigated in circulating nucleosomes by subsequent studies. Indeed, H3K9me3 and H4K20me3-related nucleosomes were suggested as CRC biomarkers (Gezer et al., 2013). Gezer et al. (2012) investigated the correlation between the H3K9me3 and H4K20me3 of cNUCs in healthy subjects and patients with colorectal cancer (CRC) and multiple myeloma and found low levels of these post-translational modifications (PTMs) in cancer. ChIP based analysis of circulating nucleosomes in serum samples by Leszinski et al. (2012b) reported a low level of H3K9me3 and H4K20me3 in patients with colorectal cancer compared to healthy control. Moreover, H3K27me3 were found to be significantly lower in CRC patients' plasma than in cancer-free individuals (Gezer et al., 2015). In summary, identification of histone PTMs isolated circulating nucleosomes have opened the possibility that blood samples collected can be used for the development of histone PTM-based cancer diagnostic marker. However, thus far, these studies are all Phase I biomarker studies. Further research is needed to determine whether any of these modifications will be clinically useful diagnostic or prognostic biomarkers in CRC. However, histone PTMs have been mostly studied for their potential as prognostic markers in this tumor type. As previously mentioned, the report of Fraga et al. (2005) was the first to strongly suggest the utility of histone PTMs in cancer diagnosis, showing loss of H4K16ac and H4K20me3 in several cancers, including CRC, and establishing these two marks as a hallmark of tumor and establishing the correlation of H4K16ac with tumor progression. Tamagawa et al. (2012) has correlated H3K4me2 and H3K9ac with the tumor histological type. In addition, lower levels of H3K4me2 and of H3K27me2 associated with poor and were found to be an independent prognostic factor. H3K36me3 correlated with lymph node metastasis (Tamagawa et al., 2013). It has also been demonstrated that PTMs can exhibit an age-dependent prognostic value in colorectal cancer (Goossens-Beumer et al., 2015), where increasing H3K27me3 and H3K9ac with advancing age was found in patients with poor survival or outcomes (death/recurrence), compared with decreasing expression in the no-event group.

More recently, combinations of modifications have been investigated in early stage colon cancer. Low nuclear expression of H3K4me3 and high expression of H3K9me3 and H4K20me3 correlated to increased survival and longer local and distant recurrence free-survival, especially in early stage 1 and 2 CRC (Bernard et al., 2014a, 2014b).

Conversely, a study of H3K27me3 and its associated polycomb proteins EZH2, BMI1 and SUZ12 in CRC indicated that an increase in the number of markers showing high expression was associated with better prognosis and revealed high expression of all four was correlated with improved overall survival and recurrence-free survival.

Benard et al. (2014c) also investigated the clinical prognostic value of the histone deacetylases SIRT1, HDAC1 and HDAC2 and the histone modifications H4K16Ac and H3K56Ac in colorectal cancer. Multivariable trend survival analyses of the combined markers showed better patient survival and less tumour recurrence when more markers showed high nuclear expression. The studied epigenetic markers showed clinical prognostic value in colorectal cancer, both as individual markers and when combined into multimarker analyses.

Enzymes involved in post-translational modifications of the histone tails may also represent potential prognostic biomarkers in CRC, as discussed in the previous section.

4. Epigenetic silencing as a therapeutic target in CRC

Reversible nature of epigenetic changes sms has drawn major attention of scientific community to study the molecular mechanism regulating the alteration in epigenetic marks. Such efforts have led to the discovery of several histone modifying enzymes and their chemical inhibitors (Cole, 2008), which has emerged as an attractive strategy in cancer treatment. Targeting these enzymes can reactivate epigenetically silenced tumor-suppressor genes by modulating the levels of histone posttranslational modifications (Espino et al., 2005). Furthermore, these drugs have also given additional advantage in the area of combinatorial chemotherapy, especially since there is growing evidence supporting the hypothesis that epigenetic alterations may be a driving force of drug resistance in human cancer (Balch and Nephew, 2013), a phenomenon that has been reported in many solid tumors, including CRC cells (Arnold et al., 2003).

Histone deacetylase inhibitors have undergone major preclinical investigations being also explored for efficacy in the treatment of various human cancers in several clinical trials. HDAC inhibitors are promising agents, as in solid

tumors they are characterized by relatively low toxicity profile and antiproliferative activities. However, unlike the earlier success in treatment of lymphomas the majority of the results among solid tumor patients have been disappointing. Indeed, side-effects and the limited clinical efficacy may result from the inability of HDACi to act as selective inhibitors (Papavassiliou and Papavassiliou, 2013).

In colorectal cancer, the current experience is mainly experimental. Histone deacetylase inhibitors are currently being admitted as monotherapy or combination therapy either with the conventional chemotherapy or with other agents. Valproic acid combined with ionization therapy may enhance tumor response. Vorinostat was the first drug of this group used in clinical trial in combination with conventional chemotherapy and managed to stabilize advanced colorectal cancer.

Interactions between different epigenetic mechanisms have led to the foundation of research on combinatorial approach of cancer treatment using epigenetic drugs. Indeed, combinations of DNA methyltransferase and histone deacetylase inhibitors appear to synergize effectively in the reactivation of epigenetically silenced genes (Cameron et al., 1999), allowing for optimal therapy. For example, experimental results show that combination therapy of vorinostat and decitabine (DNA methyl transferase inhibitor) in CRC may be more effective than treatment with a single therapeutic agent (Yang et al., 2012). Furthermore, combinatorial therapy shows promising results in overcoming resistance to conventional agents and in amplifying their effects. Various HDAC inhibitors, including the FDA-approved vorinostat, have been shown to effectively sensitize CRC cells to 5-FU-induced apoptosis (Tumber et al., 2007; Fazzone et al., 2009).

In contrast to HDACi, the application of agents targeting histone methylation, HATs and chromatin remodelers is still largely unexplored (Bardan and Liu, 2013). However, studies on histone methylation might be more suitable because of less redundancy in HMTs and HDM compared to HATs and HDACs in targeting specific amino acid residue of histone (Mack, 2010). This property of HMTs and HDMs provides exciting opportunities with more tailored treatment, while potentially minimizing side effects.

Inhibitors of EZH2, DOT1L and other HMTs have demonstrated promising therapeutic effects in preclinical CRC treatment (Huang et al., 2017). EPZ00477 is a potent inhibitor of DOT1L and resulted in inhibited sphere formation in primary colon cancer (Kryczek et al., 2014). BCI-121, which was identified as SMYD3 inhibitor by virtual screening, suppressed the growth of CRC cells (Peserico et al., 2015). Chaetocin is a fungal metabolite that inhibited the activity of SUV39H1 and the migration of CRC cells (Yokoyama et al., 2013). BIX01294 and UNC0638 are two potent and selective EHMT2 inhibitors that inhibited proliferation of CRC cell lines (Zhang et al., 2015b). DZNep is an indirect EZH2 inhibitor, which increased apoptosis in CRC cell lines and colon cancer stem cells (Benoit et al., 2013). EZH2 inhibitor GSK346 reduced migration of CRC cells (Ferraro et al., 2014). GSK126 is a highly specific inhibitor of EZH2, which resulted in reduced level of H3K27me3 (Maryan et al., 2015). AMI-1 is a PRMT5 inhibitor that inhibited proliferation of CRC cells in xenograft mouse models (Zhang et al., 2015a). Tranylcypromine, previously used as an antidepressant drug, was discovered as a potent LSD1 inhibitor, which suppressed invasion and growth of CRC cells (Ding et al., 2013). FLLL-32, one of the curcuminoids, inhibits KDM4C in vitro, resulting in the inhibition of cell proliferation of CRC cell lines (Lin et al., 2010). With another EZH2 inhibitor, EPZ-6438, entering into phase I/II trials for advanced solid tumors, histone methylation is emerging as a promising target for CRC. Development of more selective and potent small molecule modulators of histone-modifying enzymes should be emphasized in the near future.

Other drugs targeting histone modifications with therapeutic potential in CRC include statins, 5-fluorouracil, curcumin and compound K. Statins, possibly through EZH2 inhibition, suppress proliferation and induce growth arrest of CRC cells (Ishikawa et al., 2013). 5-FU induces global histone de-acetylation in multiple CRC cell lines. Du et al. (2017) identified that 5-FU reduces the binding ability of histone acetyltransferases p300 and CBP to chromatin, and induces their degradation through lysosome. Curcumin has also been identified as an inhibitor of p300 HAT, inducing p300 degradation and inhibiting the acetyltransferase activity of purified p300 (Marcu et al., 2006). Compound K, a metabolite of ginseng saponin, induces in exposed HT-29 human colon cancer cells time-dependent inhibition of histone deacetylase (HDAC) activity, mRNA and protein expression (Kang et al., 2013; Chen et al., 2016).

At the preventive level, natural compounds such as folate, epigallocatechin 3-gallate (EGCG) from green tea, genistein from soybean, catechol-containing coffee polyphenols and flavonoids seem to lower CRC risk by modulating epigenetic modifications. These compounds alter the availability of methyl groups, influence the activity of DNMTs and control DNA methylation and histone acetylation (Huang et al., 2011).

Despite of all this progress in targeting of histone PTMs in cancer patients, little is known about the utility of PTMs in monitoring the response to chemotherapy. For this purpose, levels of cNUCs and their modifications can be utilized, because circulating nucleosomes in serum are a result of apoptosis of actively dividing cells. Therefore, after

chemotherapy/radiotherapy, increase in the circulating nucleosomes correlates with progressive disease, whereas decrease was associated with disease regression in several cancers, including CRC. Nucleosome's concentration increase in serum has been shown at 24-72 h after the first application of chemotherapy and 6-24 h after the start of radiotherapy (Holdenrieder et al., 2001). Thus, indicating that nucleosomes' levels might be a useful tool for monitoring the biochemical responses during antitumor therapy, particularly for the early estimation of therapeutic efficacy.

The discovery that chromatin remodeling complexes regulate gene expression, which deregulation has been implicated in cancer has stimulated interest in screening them for small molecule regulators. However, the research on chromatin remodelers as targets for cancer therapy, including CRC, is still at a very early phase. Using different approaches, two successful screens have been performed to discover small molecule regulators for the BRG1 and RUVBL1 ATPases, which inhibited proliferation of cancer cells (Dykhuizen et al., 2012; Elkaim et al., 2012). In addition to discovering small molecules targeting the ATPase activity, resources can be invested to discover molecules to inhibit key protein–protein interactions essential for the function of chromatin remodeling complexes. The inhibition of the BAF57–AR interaction is a successful example of this strategy, in prostate cancer cells (Link et al., 2008). Additional means to inhibit chromatin remodeling complexes with this approach can also include targeting essential subunit interactions within a remodeling complex. Another strategy to therapeutically regulate chromatin remodeling is to identify small molecules that can regulate the expression of genes encoding essential subunits of chromatin remodeling complexes. This has successfully been done to reexpress the BRM ATPase in cancer cell lines that have silenced its expression without a deletion (Gramling & Reisman, 2011; Gramling et al., 2011).

5. Conclusions and future perspectives

The role of histone modifications in governing cellular functions has not yet been fully understood. However, with increased research over the past decade, the importance of chromatin environment, especially histone post-translational modifications and chromatin remodelers, in development and disease is being uncovered. Advances in our understanding of the natural history of CRC and the epigenetics of colon polyps and CRC has led to the development of epigenetic biomarker assays for CRC diagnosis, prognosis, and prediction of treatment response. The advances in understanding of the altered patterns of histone modifications and chromatin remodeling in CRC have demonstrated the potential of these altered patterns to be used as biomarkers for colon polyps and CRC. Continued investigation of these promising classes of biomarkers promises to lead to high performance assays that can be introduced into the clinical setting and used to prevent and manage patients with CRC.

Histone modifications, such as methylation and acetylation, were detected in circulating nucleosomes in blood of patients with colorectal cancer, showing considerable potential as biomarkers. The combination of several histone marks rather than single histone marks is believed to further enhance sensitivity and specificity of cancer detection. Multiplex approaches could be utilized to assess the clinical relevance of such modification patterns. Large, prospective trials are needed to show their superiority or additive value to already existing protein and DNA tumor markers.

Additionally, research in the field of histone PTMs and chromatin remodelers allows for the development of epigenetic silencing targeted therapies in CRC. Epigenetic agents, such as HDAC inhibitors, have undergone major preclinical investigations and extensive clinical trials, either alone or in combinations with conventional chemotherapeutic agents, for their efficacy to treat various types of solid cancers, including CRC. However, the current challenge is to find selective epigenetic agents that demethylate or acetylate specific target(s) or that interfere with chromatin remodelers, to reduce toxicity and improve the response to therapies.

Further work in the next decade may gain deeper understanding of the global patterns of histone posttranslational modifications and their corresponding changes which will hopefully reveal many molecular targets that can be employed as new weapons in the long fought battle against cancer.

Annex I

Table 1 - chromatin remodelers found to have an altered expression in CRC and possibly associated with tumorigenesis and, in some cases, also associated with clinical outcomes.

	Chromatin remodeler gene	Expression in CRC	Clinical correlation	Reference
Chromatin Remodelers	BRG1	increased	AJCC stage, depth of tumor invasion, distant metastasis, and histologic differentiation; more agressive, poor survival	Sanchez-Tillo et al., 2010; Watanabe et al., 2011; Shengtao et al., 2016; Zhu et al., 2016
	ARID1A	decreased	poor differentiation, lymphovascular invasion and higher pT stage	Network, 2012; Jones et al., 2012; Cajuso et al, 2014; Lee et al., 2016a; Ye et al., 2014
	SMARCC2	decreased		Kim et al., 2013
	SMARCB1	decreased	poor differentiation, liver metastasis and shorter patients' survival	Pancione et al., 2013
	CHD5	decreased		Mokarram et al., 2009; Fatemi et al., 2015
	CHD7, CHD8	decreased		Tahara et al. (2014)
	CHD4	increased	early disease recurrence and decreased overall survival	Cai et al., 2014; Xia et al., 2017
	MTA1	increased	decreased overall survival and poor clinical outcome	Higashijma et al., 2011
	SATB1	increased	poor prognosis	Mansour et al., 2016; Brocato et al., 2015
	SATB2	decreased	sensitive marker to distinguish CRC from other cancer types; reduced expression associated with poor prognosis	
	Tip60/p400	decreased	critical for the response to 5-fluorouracil; marker for the malignancy of CRC	Mattera et al., 2009; Chevillard-Briet et al., 2013
	HELLS	decreased		Choi et al., 2015
	BPTF	decreased		Lee et al., 2016b

Table 2 - histone post-translational modifications and respective regulatory enzimes found to be altered in CRC and possibly associated with tumorigenesis and, in some cases, also associated with clinical outcomes.

	Epigenetic modification/ gene of regulatory enzime	Expression in CRC	Clinical correlation	Reference
Histone acetylation	H4K16ac	decreased		Fraga et al. (2005)
	H4K12ac and H3K18ac	increased/decreased		Ashktorab et al., 2009; Nakazawa et al., 2012
	HDAC 1,2,3,7	increased/decreased		Ashktorab et al., 2009; Stypula-Cyrus et al., 2013; Ishihama et al., 2007; Rönsch et al., 2011; Stypula-Cyrus et al., 2013
	HDAC4	decreased		Bassett and Barnett, 2014
	HDAC5	increased/decreased		Bassett and Barnett, 2014; Rönsch et al., 2011; Stypula-Cyrus et al., 2013
	SIRT1	increased		Nosho et al., 2009
	p300	increased	advanced tumor stages, metastasis and poor clinical outcome; increased survival	Ishihama et al., 2007; Huh et al., 2013
	MOF	increased		Su et al., 2016
Histone methylation	H4K20me3	decrease		Fraga et al., 2005
	H3K9me2/me3	increased/decreased	lymph node metastasis, good prognosis (increase)	Yokoyama et al., 2013; Nakazawa et al., 2012; Gezer et al., 2013; Benard et al., 2014
	H3K4me3	increased/decreased		Salz et al., 2014; Lu et al., 2014
	H3K27me3	increased/decreased	poor prognosis ; metachronous liver metastasis	Gezer et al., 2015; Benard et al., 2014
	H3K79me2	increased	poor prognosis	Kryczek et al., 2014
	Bmi1	increased	advanced stages and agressive types	Du et al., 2010
	EZH2	increased		Liu et al., 2015; Fussbroich et al., 2011
	MLL2	increased	highly invasive	Kang et al, 2007
	MLL4	increased		et al. (2010)
	SETD1A	increased		Salz et al., 2014

	JARID1B	increased		Ohta et al., 2013
	LSD1	increased	correlated with TNM stage, lymph node infiltration and metastatic disease	Ding et al., 2013; Jie et al., 2013
	SUV39H1	increased		Tiwari et al., 2008
	EHMT2	increased		Zhang et al., 2015b
	KDM4B	increased	lymph node status, Duke's classification and tumor invasion	Liu et al., 2013; Berry et al., 2014
	KDM4C	increased		Kim et al., 2014
	DOT1L	increased	poor prognosis	Kryczek et al., 2014
	SMYD3	increased		Van Aller et al., 2012
	WHSC1	increased	tumor agressiveness	Hudlebusch et al., 2011
	CARM1	increased		Ou et al., 2011
	PRMT5	increased	poor patient survival	Zhang et al., 2015a
Other histone modifications	γH2AX	increased	tumor stage and perineurial invasion; poor distant metastasis-free survival and overall survival	Lee et al., 2015
	H2Bub1	decreased		Johnsen, 2012

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